

Available online at BCREC website: https://bcrec.id



Bulletin of Chemical Reaction Engineering & Catalysis, 16 (4) 2021, 857-868

Research Article

Modelling Based Analysis and Optimization of Simultaneous Saccharification and Fermentation for the Production of Lignocellulosic-Based Xylitol

Ibnu Maulana Hidayatullah¹, I Gusti Bagus Ngurah Makertihartha^{1,2}, Tjandra Setiadi¹, Made Tri Ari Penia Kresnowati^{1,3,*}

¹Department of Chemical Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung, Jalan Ganesha No. 10, Bandung, West Java, 40132, Indonesia.

²Center for Catalysis and Reaction Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung, Jalan Ganesha No. 10, Bandung, West Java, 40132, Indonesia.

³Department of Food Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung, Kampus Jatinangor, Sumedang, Indonesia.

Received: 24th July 2021; Revised: 10th September 2021; Accepted: 12nd September 2021 Available online: 20th September 2021; Published regularly: December 2021



Abstract

Simultaneous saccharification and fermentation (SSF) configuration offers efficient use of the reactor. In this configuration, both hydrolysis and fermentation processes are conducted simultaneously in a single bioreactor, and the overall processes may be accelerated. However, problems may arise if both processes have different optimum conditions, and therefore process optimization is required. This paper presents a mathematical model over SSF strategy implementation for producing xylitol from the hemicellulose component of lignocellulosic materials. The model comprises the hydrolysis of hemicellulose and the fermentation of hydrolysate into xylitol. The model was simulated for various process temperatures, prior hydrolysis time, and inoculum concentration. Simulation of the developed kinetics model shows that the optimum SSF temperature is 36 °C, whereas conducting prior hydrolysis at its optimum hydrolysis temperature will further shorten the processing time and increase the xylitol productivity. On the other hand, increasing the inoculum size will shorten the processing time further. For an initial xylan concentration of 100 g/L, the best condition is obtained by performing 21-hour prior hydrolysis at 60 °C, followed by SSF at 36 °C by adding 2.0 g/L inoculum, giving 46.27 g/L xylitol within 77 hours of total processing time.

Copyright © 2021 by Authors, Published by BCREC Group. This is an open access article under the CC BY-SA License (https://creativecommons.org/licenses/by-sa/4.0).

Keywords: lignocellulose; modelling; simultaneous saccharification and fermentation; SSF; xylitol

How to Cite: I.M. Hidayatullah, I.G.B.N. Makertihartha, T. Setiadi, M.T.A.P. Kresnowati (2021). Modelling Based Analysis and Optimization of Simultaneous Saccharification and Fermentation for the Production of Lignocellulosic-Based Xylitol. Bulletin of Chemical Reaction Engineering & Catalysis, 16(4), 857-868 (doi:10.9767/bcrec.16.4.11807.857-868)

Permalink/DOI: https://doi.org/10.9767/bcrec.16.4.11807.857-868

1. Introduction

Xylitol is a pentitol or polyalcohol sugar $(C_5H_{12}O_5)$ commonly used in the pharmaceutical

sector as a dental remineralizing agent. Xylitol can increase salivation and inhibit the cellular activity of cariogenic organisms, thus reducing plaque, gum swelling, dental erosion and preventing xerostomia (lack of saliva production) [1–4]. In addition, xylitol can be used as a sweetener; it is commonly used in food indus-

* Corresponding Author.

 $Email: kresnowati@che.itb.ac.id (M.T.A.P. Kresnowati); \\ Telp: +62-812-2028-878, Fax: +62-22-2501438$

tries and is categorized as safe for diabetics [5–8]. Other than that, xylitol is used as a building block for ethylene glycol and propylene glycol formation using Ruthenium or copper as the catalyst in the hydrogenolysis process [9,10] and as a building block for 2,3,4,5 tetrahydroxypentanoic acid and xylonic acid using diperiodatoargentate(III) and Ru(III) as the catalyst through oxidation [11,12].

Conventionally, xylitol production involves the hydrogenation of xylose from lignocellulosic biomass. Lignocellulose-based materials are pretreated and hydrolyzed using dilute sulfuric acid, after which the hydrolyzate is purified using chromatography to obtain xylose. Pure xylose solution is then catalytically hydrogenated using Raney-Nickel or Ru/C (Rutheniumcarbon) as the catalysts become xylitol [13,14]. However, this conventional xylitol production method has several disadvantages: the process uses much energy as it is conducted at relatively high pressure and temperature (50–60 bar and 140–200 °C); the process requires delicate purification of the hydrolysate to obtain pure xylose; and need more investment in types of equipment, numerous intermediate purification, product recovery, catalyst deactivation, and recycling process [14-16]. An alternative method of producing xylitol from lignocellulosebased materials involves a bioprocessing system. It includes enzymatic hydrolysis using xylanase to obtain xylose containing hydrolysate and microbial fermentation to convert xylose in hydrolysate into xylitol [15,17,18]. However, the hydrolysis and fermentation processes are generally conducted in different reactors, better known as separate hydrolysis and fermentation (SHF), because they have different operating conditions. As a result, the fermentative sugar production process requires a longer processing time and is still considered uneconomical.

Alternatively, the bioprocess route for xylitol production, namely, hydrolysis and fermentation, can be conducted simultaneously or better known as simultaneous saccharification and fermentation (SSF). In principle, both the hydrolysis and fermentation processes coincide in the same reactor, providing direct utilization of hydrolysis product, the monomeric sugar, as the carbon source for the fermenting agent of the desired product [18-20]. Another advantage of the SSF technique is to enhance productivity in less time [18,21]. Consequently, both processes are conducted at the same operating condition. This operating condition is an issue within itself, given that the hydrolysis/saccharification process necessitates more heat than the fermentation process. If the process is allowed, the fermenting agent will perish, and no xylitol will be produced. Moreover, the application SSF process from lignocellulose-based materials minimizes the potential substrate inhibition on the fermentation process and the potential product inhibition on the enzymatic hydrolysis process, increasing the yield and productivity [20,22,23]. Based on these considerations, optimal temperature management is required to ensure the SSF process's continuation.

Previous studies showed that SSF combined with prior hydrolysis in ethanol production increased the ethanol yield than the SSF only [21,24]. Burhan et al. [18] reported the implementation of SSF for xylitol production from oil palm empty fruit bunch (OPEFB). In their research, the duration of the prior hydrolysis process was varied to achieve the optimum results. Overall, at the same total processing time, up to a 4-fold increase of the xylitol yield from OPEFB compared with SHF was obtained [18]. Prior hydrolysis is necessary to provide sufficient substrate for initializing the fermentation. However, the optimum temperature for conducting hydrolysis and fermentation simultaneously has been overlooked; the SSF was conducted at the optimum fermentation temperature, leading to low enzymatic hydrolysis activity. The optimum temperature of xylan hydrolysis using xylanase is reported in the range of 40-70 °C [25,26], whereas the optimum temperature for xylitol-producing yeast fermentation is reported in 10–44 °C [27–30]. Conducting the SSF at the optimum temperature for both hydrolysis and fermentation would significantly increase the process performance. The temperature setting for SSF and the duration of the prior hydrolysis process can be optimized further to higher xylitol productivity.

A predictive model may serve as a useful tool to explore the interaction between parameters without conducting wet experiments. In particular, kinetic modelling can be applied to search for the optimal SSF configuration: the operating temperature, the duration of the prior hydrolysis, or the initialization of the fermentation-based on a particular initial cell concentration. This paper presents the development of a kinetic model for lignocellulosic material-based xylitol production using SSF. The model was further used for thoroughly studying the effect of process temperature, prior hydrolysis, switching time (the start of SSF), and the inoculum size to estimate xylitol concentration and its productivity. Therefore, the results could be applied on a laboratory level and

eventually developed on both a pilot and an industrial scale.

2. Materials and Method

The model was built by assuming that the process took place in a single batch reactor. The xylan-based hemicellulose in lignocellulosic material was pretreated before being put into the reactor for enzymatic hydrolysis and fermentation. Xylitol production through the bioprocessing pathway is shown in Figure 1. During the hydrolysis process, xylan was hydrolyzed into xylose. The yeast further utilized xylose as the carbon source for biomass growth and xylitol formation during the fermentation process. The maximum theoretical yield of xylose is 1.14, whereas the theoretical maximum yield of xylitol is 0.9 [31,32]. This model assumed that xylitol was the only metabolite product produced during the fermentation and that a decrease in cell concentration was neglected. Overall, the mass balances describing the process are presented in Equations (1)–(4).

$$\frac{dC_{xylan}}{dt} = -r_{hyd} \tag{1}$$

$$\frac{dC_{xylose}}{dt} = 1.14 \times r_{hyd} Y_{C_{xylose}/C_{xylan}} - r_{biomass} \frac{1}{Y_{X/C_{xylose}}}$$
(2)

$$\frac{dC_X}{dt} = r_{biomass} \tag{3}$$

$$\frac{dC_{xylitol}}{dt} = r_{xylitol} \tag{4}$$

2.1 Kinetic Model Developments

2.1.1 Xylan hydrolysis

The hydrolysis rate process (r_{hyd}) was modelled using the Michaelis Menten reaction kinetics equation presented in Equation (5). With $r_{max,hyd}$ (g/(L,h)) is the maximum rate of hydrolysis, C_{xylan} is the concentration of xylan (g/L), and $K_{m,hyd}$ is the Michaelis constant for the xylan hydrolysis (g/L) [13].

$$r_{hyd} = \frac{r_{max,hyd} \times C_{xylan}}{K_{m,hvd} + C_{xvlan}}$$
(5)

Like other chemical reactions, a higher temperature can increase the rate of enzymatic reactions. However, higher temperature also raises the rate of thermal denaturation and the loss of biocatalyst activity [33,34]. Within the range of 40–60 °C, however, the overall rate of xylan hydrolysis using xylanase is still increasing with temperature [25], and the overall effect of temperature on this enzymatic reaction can be modelled following the Arrhenius equation as is presented in Equation (7):

$$r_{\max,hyd} = k_{hyd} \times E_0 \tag{6}$$

$$k_{hyd} = A_{hyd} \times e^{-\frac{E_{a,hyd}}{RT_{hyd}}} \tag{7}$$

Where, E_{θ} is the concentration of the initial concentration of enzyme used (g/L), k_{hyd} is the catalytic constant of hydrolysis (h⁻¹), A_{hyd} is the Arrhenius constant for the hydrolysis reaction (h⁻¹), $E_{a,hyd}$ is the activation energy of the hydrolysis reaction (kJ/mol), R is the universal gas constant (kJ/mol.K), and T_{hyd} is the temperature of hydrolysis (K).

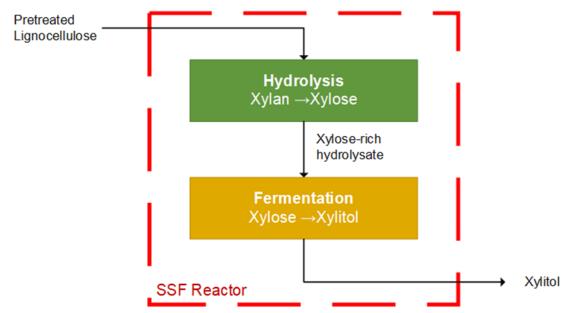


Figure 1. Bioprocess-based xylitol production.

Copyright © 2021, ISSN 1978-2993

2.1.2 Biomass growth

The biomass growth rate is defined following the first-order reaction kinetics with respect to the biomass concentration (C_X , g/L) (Equation (8)). Whereas, the biomass-specific growth rate (μ , h^{-1}) is defined following the Monod equation that correlates the specific growth rate with substrate (xylose) concentration (C_{xylose} , g/L).

$$r_{biomass} = \mu \times C_X \tag{8}$$

$$\mu = \frac{\mu_{max,fer} \times C_{xylose}}{K_{s,fer} + C_{xylose}}$$
(9)

In which, $K_{s,fer}$ is the growth saturation constant on xylose (g/L) and $\mu_{max,fer}$ is the maximum specific growth rate of the fermentation process (h⁻¹). Further, the effect of temperature of biomass growth can be modelled following Sanchéz *et al.* [28] as:

$$\mu_{max,fer} = A_{fer} \times e^{-\frac{E_{g,fer}}{RT}} - B_{fer} \times e^{-\frac{E_{d,fer}}{RT}}$$
(10)

where, $E_{g,fer}$ is the cell activation energy for growth (kJ/mol), $E_{d,fer}$ is the deactivation energy when the cell has entered the death phase (kJ/mol), A_{fer} is the cell activation coefficient (h⁻¹), B_{fer} is the cell inactivation coefficient (h⁻¹). Other effects of microenvironment conditions, such as the oxygen concentration in the fermentation broth or the acidity level of the media, were not considered in this model. Combination of Equations (8), (9), and (10) are given as follows:

$$r_{biomass} = \left(\frac{\left(A_{fer} \times e^{\frac{E_{g,fer}}{RT}} - B_{fer} \times e^{\frac{E_{d,fer}}{RT}}\right) \times C_{xylose}}{K_{s,fer} + C_{xylose}}\right) \times C_{X}$$
(11)

2.1.3 Xylitol formation

Xylitol production rate is modelled using the growth-associated product. The equation is approached as follows [33]:

$$r_{xylitol} = \mu \times C_X \times Y_{C_{xylitol}/X}$$
 (12)

A combination of Equations (9), (10), and (12) are given as follows.

$$r_{xylitol} = \left(\frac{\left(A \times e^{\frac{E_g}{RT}} - B \times e^{\frac{E_d}{RT}}\right) \times C_{xylose}}{K_{s,fer} + C_{xylose}}\right) \times C_X \times Y_{C_{xylitol}/X}$$
(13)

where, $Y_{Cxylitol/X}$ is the yield of xylitol formed from biomass activity.

2.1.4 Determination of cell and xylitol productivity

After the xylose was converted entirely, we could measure how much cell and xylitol productivity was obtained in each configuration process. The cell productivity is determined as follows (Equation (14)).

$$Q_X = \frac{C_X}{t_p} \tag{14}$$

where, Q_X and t_p are cell productivity (g/(L.h)) and total processing time (h), respectively. Also, xylitol productivity is defined as follows:

$$Q_{xylitol} = \frac{C_{xylitol}}{t_p} \tag{15}$$

where, Q_X is the xylitol productivity (g/(L.h)).

2.2 Model Simulation, Model Assumptions, and Boundary Conditions

The simulation started with the hydrolysis of xylan to xylose, followed by the fermentation of the xylose to xylitol. The initial concentration of xylan was set at 100 g/L. Unless stated otherwise, the initial biomass concentration of 0.5 g/L was introduced at the beginning of the fermentation process. The maximal total process time was set to be 200 hours.

The pretreatment of lignocellulosic material generates several derivative compounds that disrupt the saccharification and fermentation processes [35-38]. To avoid inhibition of both processes, we used pure xylan as an assumption. Moreover, all simulation processes were stopped when the xylitol reached the maximum concentration in each process, and the other compounds were considered not to interfere with the process. Particularly for Separate Hydrolysis and Fermentation (SHF), hydrolysis was halted if 1% of xylan residue was obtained. Thus, during hydrolysis, the xylobiose and other xylooligosaccharide formations were neglected. Moreover, the xylitol was assumed not consumed by cells or converted into the other compounds so that xylitol accumulation has decreased. The simulations were conducted using the Matlab R2018a. The supporting data for simulations are shown in Table 1.

3. Results and Discussion

3.1 Mapping the Effects of Temperature on Hydrolysis and Fermentation

The literature study indicated that the hydrolysis of xylan and the fermentation for xylitol production occurred in the different temperature ranges. For example, Mardawati *et al.*

[25] and Meilany et al. [26] reported the optimum temperature for xylan hydrolysis for obtaining the highest xylose yield at 60 °C. In contrast, Pappu and Gummadi [37] and Sánchez et al. [28,39] reported the optimum temperature for fermentation at 35 °C. Indeed, a previous study showed that the range temperature for the high biomass growth and xylitol productivity was 30-35 °C [18,30,40]. Burhan et al. [18] reported a decrease in fermentation xylitol performance when the temperature was increased from 30 to 37 °C. The mapping of the effects of temperature on hydrolysis and fermentation, particularly the maximum rate of hydrolysis (k_{hyd}) and the maximum specific growth rate of biomass (μ_{max}) , in the temperature range of 25–60 °C are presented in Figure 2.

Within the temperature range of 25–60 °C, the xylan hydrolysis rate was shown to increase along with an increase in temperature, whereas three distinct trends of fermentation

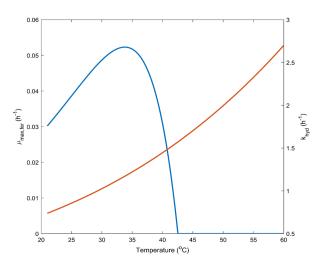


Figure 2. Effect of temperature on hydrolysis and fermentation processes; the blue line (—) denotes the fermentation model; the red line (—) represents the hydrolysis model.

rate were observed. First, an increasing trend of the maximum specific biomass growth rate was observed between 25–34 °C. Second, between 34 °C and 42 °C, a decreasing trend of the maximum specific biomass growth rate was observed. Finally, the biomass could not grow above 42 °C. Therefore, the optimum condition for both hydrolysis and fermentation were expected to be in the range of 25–42 °C. In this condition, both fermentation and hydrolysis can proceed, although not in each optimum condition.

3.2 Separate Hydrolysis-Fermentation (SHF)

Xylitol production via the SHF system was simulated as the reference. The SHF was conducted at the optimum temperature for each process, as calculated in the previous section (Figure 2). During this simulation, the hydrolysis was set to proceed at 60 °C, whereas the following fermentation proceeded at 34 °C. The temperature switch was assumed to occur in-

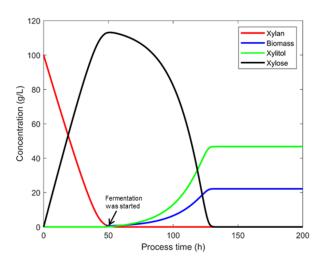


Figure 3. Xylitol production by using the SHF method, both the hydroylysis and the succeeding fermentation were conducted at their optimum temperatures.

Table 1. Supporting data for modeling and simulation of xylitol production.

Parameter	Value	Reference
$K_{m,hyd}$ (g/L)	6.896	[46]
$Y_{X/Cxylose}$ (g/L)	0.190	[47]
$Y_{Cxylitol/Cxylose}$ (g/L)	0.41	[47]
$K_{s,fer}$ (g/L)	5.897	[46]
$E_{a,hyd}$ (kJ/mol)	27.08	[48]
R (J/mol.K)	8.314	[33]
A_{hyd}	47786.71	[48]
$E_{g,fer}$ (kJ/mol)	56.9	[28]
$E_{d,fer}$ (kJ/mol)	138.9	[28]
A_{fer} (1/h)	4.32×10^{8}	Obtained from data [28]
B_{fer} (1/h)	1.62×10^{22}	Obtained from data [28]

stantly. Fermentation was initiated shortly after the completion of xylan hydrolysis, which was at 99% xylan conversion. The results of the simulation of xylitol production using the SHF method are shown in Figure 3.

Figure 3 shows that at 48 hours, 99% of xylan has been converted, giving the xylose concentration of 112.68 g/L and a residual xylan of 1.16 g/L. The fermentation was then initiated at that time, and the simulation was continued until a total processing time of 200 hours. Xylose was consumed slowly by the biomass for the first 32 hours of fermentation. At that time, the biomass was in the lag phase, and xylitol was formed slowly. After 80 hours of processing time, a fast decrease in xylose concentration was observed, indicating high xylose consumption for growth and xylitol production. The final concentration of biomass and xylitol obtained in the simulation was 21.85 g/L and 46.07 g/L, successively, which were achieved at the 6th day or 128 hours of processing time. The final xylitol concentration was the maximum concentration that could be achieved based on theoretical yield.

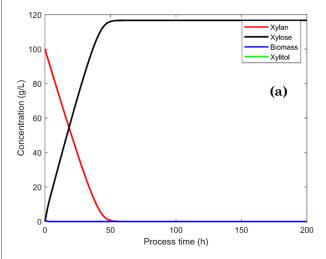
Dominguez et al. [40] reported that the xylitol production using 120 g/L synthetic substrates using 1.2 g/L initial yeast concentration gave xylitol and yeast concentration near 80 g/L and 5 g/L, respectively, after 72-hour fermentation. The fermentation was conducted at the optimal fermentation temperature, and all xylose was utilized within the observed fermentation time. This reported fermentation time was comparable to the time required for consuming all xylose in the fermentation simulation, 80 hours (Figure 3).

3.3 Simultaneous Saccharification and Fermentation (SSF)

During SSF, all components of the process: xylan as the substrate, the xylanolytic enzyme, and the biomass inoculum (as the fermenting agent) were present in the bioreactor, such that the hydrolysis and fermentation coincided. Therefore, two distinct strategies were evaluated: conducting SSF at the optimum hydrolysis temperature (60 °C) and conducting the SSF at the optimum fermentation temperature (34 °C). The simulation results are presented in Figure 4. In addition, the model was also run for conducting SSF at 30 °C and at initial xylan concentration of 11.25 g/L such the obtained results could be compared with literature data [18], for model verification.

At the optimum hydrolysis temperature of 60 °C (Figure 4(a)), xylan was converted into xylose, increasing the xylose profile until 48-hours. However, the following fermentation could not proceed as the fermenting agent of xylitol production, such as *Debaromyces hansenii*, *Debaromyces nepalensis*, *Pachysolen tannophilus*, or *Candida tropicalis*, could only grow in the temperature range of 15–40 °C [28–30,41,42]. Thus, thereby no biomass growth nor product formation is observed (Figure 2). By the end of this simulation, only as much as 115.31 g/L of xylose was formed.

At the optimum fermentation temperature of 34 °C (Figure 4(b)), hydrolysis proceeded slowly. Xylan was slowly hydrolyzed and would be hydrolyzed entirely at 110 hours. Although the biomass inoculum was already present from the beginning of SSF, the low xylose concentration led to slow biomass growth.



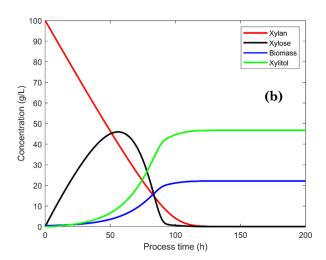


Figure 4. Xylitol production via SSF method conducted at (a) the optimum hydrolysis temperature and (b) the optimum fermentation temperature.

Significant biomass concentration was only observed after 43 hours, and the biomass reached the stationary phase when the substrate was exhausted. Xylose was entirely utilized at 109 hours, resulting in xylitol and biomass concentrations of 46.06 g/L and 21.83 g/L, respectively. The final xylitol concentration was the maximum concentration that could be achieved based on theoretical yield.

Nevertheless, compared to the SHF process (and assuming that the working volume of the SSF is the same as SHF), it generates a similar total mole of xylitol. On the other hand, the SSF method proceeded faster to achieve the same xylitol concentration. Therefore, applying the optimum fermentation temperature is preferable to the optimum hydrolysis temperature in SSF.

The hydrolysis proceeded even slower at 30 °C, and thereby the overall process proceeded more slowly. The maximum xylitol concentration was eventually achieved at 104 hours, giving xylitol productivity of x g/L/h. When compared to literature data [18], the SSF experiment conducted by Burhan et al at similar operating condition for 120 hours resulted in xylitol productivity of 0.006 g/L/h. Our model simulation gave slightly higher value than experimental data. It is important to note that the experiment was conducted by using pretreated Oil Palm Empty Fruit Bunches as the substrate in which some inhibitory substances might be formed during the substrate preparation that led to lower overall xylitol productivity.

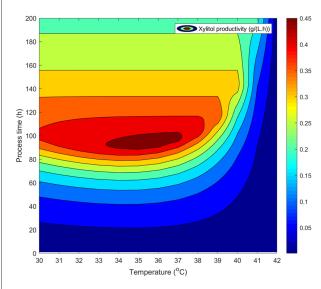


Figure 5. The results of SSF simulation for contour map showing temperature, process time, and xylitol productivity.

3.4 Simultaneous Hydrolysis-Fermentation at Optimum SSF Temperature

In determining the optimum temperature for SSF, simulations were conducted within the temperature range of 30–43 °C using Equations (7), (11), and (13) for temperature-dependent hydrolysis, biomass growth, and xylitol formation, respectively. Figure 5 showed the contour plot between temperature, the processing time, and the resulting xylitol productivity. The lowest to the highest xylitol productivity was represented by the dark blue to dark red contour area.

The maximum xylitol concentration can always be achieved at various SSF temperatures. However, the processing time required to achieve the maximum xylitol concentration varied with temperature, leading to a variation in the xylitol productivity. The low enzymatic activity at the range of temperature of 30-43 °C resulted in slow xylose accumulation. The low xylose concentration led to slow biomass growth and xylitol formation. For total processing time under 60 hours, low xylitol productivity (<0.15 g/(L.h)) was observed at the simulated temperature range (Figure 5). An increasing trend of xylitol productivity was observed at processing time 60-100 hours. However, the xylitol productivity slowly decreased after 100 hours of processing time. The optimum xylitol productivity was achieved in the temperature range of 34-37 °C, marked by the dark red region in Figure 5. At 36 °C, the maximum xylitol concentration was achieved at 102 hours, gave the highest xylitol productivity of 0.45 g/(L.h). Conducting SSF at this optimum SSF temperature, 36 °C, led to higher xylitol productivity than conducting SSF at the optimum fermentation temperature.

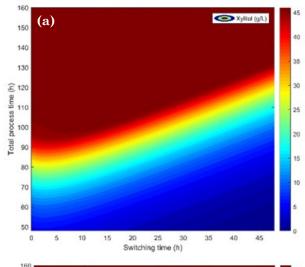
3.5 Semi-Simultaneous Hydrolysis-Fermentation and the Effect of Initial Cell Concentration

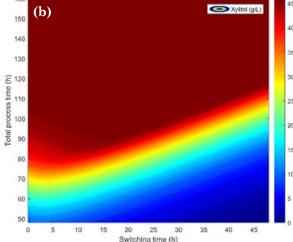
The performance of SSF may be improved by conducting a prior hydrolysis process, at the optimized temperature for hydrolysis, before the initiation of SSF. The overall process, the combination of the prior hydrolysis process and the SSF process, is called semi-simultaneous hydrolysis and fermentation (semi-SSF). In practice, the initiation of SSF can be set by adding biomass inoculum to various concentrations. This event will be referred to as the switching time in the remaining discussion.

The determination of the optimum switching time was conducted by varying the duration of prior hydrolysis, ranging between 0 to

48 hours, at a particular initial cell concentration. The results are shown in Figure 6, with the dark blue to dark red colour denotes the lowest to highest xylitol concentration, respectively.

In general, an increase in the switching time resulted in a longer total processing time required to achieve the maximum xylitol concentration (Figure 6). The best configuration was obtained by prior hydrolysis time of 8 hours led to a total processing time of 93 hours to achieve the maximum xylitol concentration (Figure 6(a)). The later the switching time, the lower the ability of the biomass to ferment so





that productivity decreases. The obtained results are consistent with a previous study conducted by Burhan *et al.* [18], in which prior hydrolysis resulted in higher xylitol concentration and productivity.

The overall processing time could be further improved by increasing the initial biomass concentration for the fermentation, or in other words, increasing the inoculum size added to the system (Figure 6(b-c)). Table 2 shows the effects of initial cell concentrations on cell and xylitol productivities. In addition, the increase in the initial cell concentrations shortened the total processing time despite longer prior hydrolysis time. These results showed that the fermentation process was the limiting factor of xylitol production. Increasing the concentration of cell inoculum is thus recommended to increase xylitol productivity and shorten the total process time.

3.6 Comparison of Configurations of All Processes

Various process configurations for xylitol production have been simulated. We summarized the effect of process configuration and process temperature on overall processing time, cell, and xylitol productivities at the same initial xylan and cell concentration (100

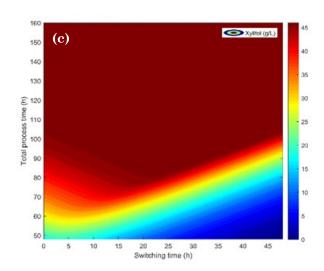


Figure 6. Contour map showing the effect of switching time to xylitol concentration at initial cell concentration of (a) 0.5 g/L; (b) 1 g/L; and (c) 2 g/L.

Table 2. The effect of initial cell concentration in the inoculum to SSF for xylitol production.

Initial cell concentration (g/L)	Cell productivity (g cell/(L.h))	Xylitol productivity (g xylitol/(L.h))	Optimum Prior hydrolysis time (hour)	Overall processing time (hour)
0.5	0.221	0.496	8	93
1	0.263	0.550	14	84
2	0.307	0.599	21	77

g/L and 0.5 g/L). The summary of cell and xylitol productivity and the time required to achieve the maximum xylitol concentration are shown in Table 3. SSF at optimum condition temperature provided better cell and xylitol productivities than SSF at the optimum fermentation temperature. SSF with high initial cell concentration increased the cell and xylitol productivities further. The best SSF configuration obtained in the simulations was 21 hours before hydrolysis at the optimum hydrolysis temperature, followed by SSF at 36 °C by adding a cell inoculum up to 2 g/L. It is shortened the overall processing time to achieve the maximum xylitol concentration to 77 hours, or 39.84% compared with the SHF. The overall processing time for SHF was 128 hours, whereas the overall processing time for the best SSF configuration was 77 hours.

The obtained results confirmed previous results of Burhan et al. [18] and Öhgren et al. [45], which produced xylitol and ethanol using the SHF and SSF methods. In addition, they obtained higher productivity results when using the SSF configuration. The SSF simulation results showed that the optimum temperature, intermediate xylose formation-reduction, xylitol formed, and biomass growth could be predicted adequately. Experimental validation through testing the SSF temperature and the pre-hydrolysis time could be conducted further to confirm the accuracy of the used model parameters.

Overall, the developed kinetic model simulation has been applied to design the configuration and the SSF operation process. However, the model could be further improved by incorporating non-ideal conditions such as the inhibitory term to the hydrolysis process [35] or the inhibitory term to the xylitol fermentation pro-

cess [6,37,38], giving a more accurate estimation of the SSF process of lignocellulosic material. In addition, detailed kinetics of the related process and the estimated concentration of the inhibitory substance in a specific process, for example, SSF of OPEFB, needed to be defined. Nonetheless, this paper showed that SSF or semi SSF is an alternative process configuration that led to higher product (xylitol) productivity.

4. Conclusions

The kinetic model describing the SSF for xyproduction from hemicellulose lignocellulosic material has been successfully developed and simulated. Our simulation showed that the performance of the SSF process was affected by the process temperature, the length of prior hydrolysis or the switching time, and the initial biomass concentration. Overall, it was concluded that the SSF configuration led to higher xylitol productivity than the SHF. The best SSF configuration was the combination of prior hydrolysis at the optimum hydrolysis temperature for 21 hours (semi SSF), SSF temperature of 36 °C, and initial biomass concentration of 2 g/L, which then led to an increased cell and xylitol productivity to 0.307 and 0.599 g/(L.h), respectively.

Acknowledgments

We would like to acknowledge the Ministry of Research, Technology and Higher Education (Kemenristekdiki) Republic of Indonesia under the Program Magister Doktor untuk Sarjana Unggul (PMDSU) for research funding support (grant numbers 0017y/I1.C06/PL/2019). Also, we would like to thank Aulia Maulana for assisting in the model and simulation.

Table 3	Regulte of	all conf	igurations of	f nrocassas o	f vylital	production.
Table 5.	. Itesuits oi	an com.	iguramons oi	. กากกลอยคอ ก	ι ανμισι	broauchon.

Process configuration	Temperature (°C)	Cell productivity (g-cell/(L.h))	Xylitol productivity (g-xylitol/(L.h))	Total process time (hour)
SHF	Hydrolysis 60 Fermentation 34	0.176	0.360	128
SSF at optimum hydrolysis temperature	60		-	48
SSF at optimum fermentation temperature	34	0.202	0.423	109
SSF at optimum condition temperature	36	0.214	0.452	102
Semi SSF with 8-hour pre- hydrolysis	Pre-hydrolysis 60 SSF 36	0.221	0.496	93
Semi SSF with 21-hour prehydrolysis, with 2 g/L initial biomass concentration	Pre-hydrolysis 60 SSF 36	0.307	0.599	77

References

- [1] Nayak, P.A., Nayak, U.A., Khandelwal, V. (2014). The effect of xylitol on dental caries and oral flora. *Clin. Cosmet. Investig. Dent.*, 6, 89–94. DOI: 10.2147/CCIDE.S55761
- [2] Mäkinen, K.K. (2010). Sugar Alcohols, Caries Incidence, and Remineralization of Caries Lesions: A Literature Review. *Int. J. Dent.*, 2010, 1–23. DOI: 10.1155/2010/981072
- [3] Ramesh, S., Muthuvelayudham, R., Rajesh Kannan, R., Viruthagiri, T. (2013). Enhanced production of xylitol from corncob by Pachysolen tannophilus using response surface methodology. *Int. J. Food Sci.*, 2013, 1-8. DOI: 10.1155/2013/514676
- [4] Aranda-Barradas, J.S., Garibay-Orijel, C., Badillo-Corona, J.A., Salgado-Manjarrez, E. (2010). A stoichiometric analysis of biological xylitol production. *Biochem. Eng. J.*, 50, 1–9. DOI: 10.1016/j.bej.2009.10.023
- [5] Ur-Rehman, S., Mushtaq, Z., Zahoor, T., Jamil, A., Murtaza, M.A. (2015). Xylitol: A Review on Bioproduction, Application, Health Benefits, and Related Safety Issues. Crit. Rev. Food Sci. Nutr., 55, 1514–1528. DOI: 10.1080/10408398.2012.702288
- [6] Zhang, J., Geng, A., Yao, C., Lu, Y., Li, Q. (2012). Effects of lignin-derived phenolic compounds on xylitol production and key enzyme activities by a xylose utilizing yeast Candida athensensis SB18. *Bioresour. Technol.*, 121, 369-378. DOI: 10.1016/j.biortech.2012.07.020
- [7] Anil, N., Sudarshan, K., Naidu, N.N., Ahmed, M. (2016). Production of Xylose from Corncobs. Int. J. Eng. Res. Appl., 6, 77–84.
- [8] Cortez, D.V., Roberto, I.C. (2010). Improved xylitol production in media containing phenolic aldehydes: Application of response surfacemethodology for optimization and modeling of bioprocess. J. Chem. Technol. Biotechnol., 85, 33–38. DOI: 10.1002/jctb.2265
- [9] Sun, J., Liu, H. (2011). Selective hydrogenolysis of biomass-derived xylitol to ethylene glycol and propylene glycol on supported Ru catalysts. *Green Chem.*, 13, 135–142. DOI: 10.1039/c0gc00571a
- [10] Huang, Z., Chen, J., Jia, Y., Liu, H., Xia, C., Liu, H. (2014). Selective hydrogenolysis of xylitol to ethylene glycol and propylene glycol over copper catalysts. Appl. Catal. B Environ., 1 4 7 , 3 7 7 - 3 8 6 . D O I : 10.1016/j.apcatb.2013.09.014
- [11] Gowda, J.I., Nandibewoor, S.T. (2012). Mechanism of oxidation of xylitol by a new oxidant, diperiodatoargentate (III), in aqueous alkaline medium. Synth. React. Inorganic, Met. Nano-Metal Chem., 42, 1183–1191. DOI: 10.1080/15533174.2012.684261

- [12] Srivastava, A., Bansal, S. (2015). Kinetics and Mechanism of Ru(III) Catalysed Oxidation of Xylitol by Chloramine-T in Perchloric Acid Medium. *International Journal of Chemical and Physical Sciences*, 4, 39–48.
- [13] Baudel, H.M., de Abreu, C.A.M., Zaror, C.Z. (2005). Xylitol production via catalytic hydrogenation of sugarcane bagasse dissolving pulp liquid effluents over Ru/C catalyst. J. Chem. Technol. Biotechnol., 80, 230–233. DOI: 10.1002/jctb.1155
- [14] Delgado Arcaño, Y., Valmaña García, O.D., Mandelli, D., Carvalho, W.A., Magalhães Pontes, L.A. (2020). Xylitol: A review on the progress and challenges of its production by chemical route. Catal. Today., 344, 2–14. DOI: 10.1016/j.cattod.2018.07.060
- [15] Rafiqul, I.S.M., Sakinah, A.M.M. (2013). Processes for the Production of Xylitol-A Review. Food Rev. Int., 29, 127–156. DOI: 10.1080/87559129.2012.714434
- [16] Dasgupta, D., Bandhu, S., Adhikari, D.K., Ghosh, D. (2017). Challenges and prospects of xylitol production with whole cell biocatalysis: A review. *Microbiol. Res.*, 197, 9– 21. DOI: 10.1016/j.micres.2016.12.012
- [17] Kresnowati, M., Mardawati, E., Setiadi, T. (2015). Production of Xylitol from Oil Palm Empty Friuts Bunch: A Case Study on Bioefinery Concept. *Mod. Appl. Sci.*, 9, 206. DOI: 10.5539/mas.v9n7p206
- [18] Burhan, K.H., Kresnowati, M.T.A.P., Setiadi, T. (2019). Evaluation of simultaneous saccharification and fermentation of oil palm empty fruit bunches for xylitol production. Bull. Chem. React. Eng. Catal., 14, 559–567. DOI: 10.9767/bcrec.14.3.3754.559-567
- [19] Wyman, C.E., Spindler, D.D., Grohmann, K. (1992). Simultaneous saccharification and fermentation of several lignocellulosic feedstocks to fuel ethanol. *Biomass and Bioenergy*, 3, 301–307. DOI: 10.1016/0961-9534(92)90001-7
- [20] Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., Cantarella, M. (2000). Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw. J. Ind. Microbiol. Biotech., 25, 184–192. DOI: 10.1038/sj.jim.7000054
- [21] Shen, J., Agblevor, F.A. (2010). Modeling semi-simultaneous saccharification and fermentation of ethanol production from cellulose. *Biomass and Bioenergy*, 34, 1098–1107. DOI: 10.1016/j.biombioe.2010.02.014

- [22] Ballesteros, M., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, I. (2004). Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces marxianus CECT 10875. Process Biochem., 39, 1843–1848. DOI: 10.1016/j.procbio.2003.09.011
- [23] Tomás-Pejó, E., Oliva, J.M., Ballesteros, M., Olsson, L.: Comparison of SHF and SSF processes from steam-exploded wheat straw for ethanol production by xylose-fermenting and robust glucose-fermenting Saccharomyces cerevisiae strains. *Biotechnol. Bioeng.*, 100, 1122–1131. DOI: 10.1002/bit.21849
- [24] Gonçalves, F.A., Ruiz, H.A., Nogueira, C.D.C., Dos Santos, E.S., Teixeira, J.A., De Macedo, G.R. (2014). Comparison of delignified coconuts waste and cactus for fuel-ethanol production by the simultaneous and semisimultaneous saccharification and fermentation strategies. Fuel, 131, 66-76. DOI: 10.1016/j.fuel.2014.04.021
- [25] Mardawati, E., Werner, A., Bley, T., Kresnowati, M.T.A.P., Setiadi, T. (2014). The Enzymatic Hydrolysis of Oil Palm Empty Fruit Bunches to Xylose. J. Japan Inst. Energy., 93, 973–978. DOI: 10.3775/jie.93.973
- [26] Meilany, D., Mardawati, E., Tri, M., Penia, A., Setiadi, T. (2017). Kinetic Study of Oil Palm Empty Fruit Bunch Enzymatic Hydrolysis. *Reaktor*, 17, 197–202. DOI: 10.14710/reaktor.17.4.197-202
- [27] Sampaio, F.C., De Moraes, C.A., De Faveri, D., Perego, P., Converti, A., Passos, F.M.L. (2006). Influence of temperature and pH on xylitol production from xylose by Debaryomyces hansenii UFV-170. Process Biochem., 41, 675-681. DOI: 10.1016/j.procbio.2005.08.019
- [28] Sánchez, S., Bravo, V., Moya, A.J., Castro, E., Camacho, F. (2004). Influence of temperature on the fermentation of D-xylose by Pachysolen tannophilus to produce ethanol and xylitol. *Process Biochem.*, 39, 673–679. DOI: 10.1016/S0032-9592(03)00139-0
- [29] Gummadi, S.N., Kumar, S. (2008). Effects of temperature and salts on growth of halotolerant Debaryomyces nepalensis NCYC 3413. American Journal of Food Technology, 3(6), 345-360. DOI: .10.3923/ajft.2008.354.360
- [30] Mohamad, N.L., Mustapa Kamal, S.M., Gliew, A. (2009). Effects of temperature and pH on xylitol recovery from oil palm empty fruit bunch hydrolysate by Candida tropicalis. *Journal of Applied Sciences*, 9(17), DOI: 10.3923/jas.2009.3192.3195

- [31] Barbosa, M.F.S., de Medeiros, M.B., de Mancilha, I.M., Schneider, H., Lee, H. (1988). Screening of yeasts for production of xylitol fromd-xylose and some factors which affect xylitol yield inCandida guilliermondii. *J. Ind. Microbiol.*, 3, 241–251. DOI: 10.1007/BF01569582
- [32] Gulati, M., Kohlmann, K., Ladisch, M.R., Hespell, R., Bothast, R.J. (2996). Assessment of ethanol production options for corn products. *Bioresour. Technol.*, 58, 253–264. DOI: 10.1016/S0960-8524(96)00108-3
- [33] Shuler, M.L., Kargi, F. (2002). *Bioprocess Engineering Basic Concepts*. Prentice hall PTR, New Jersey (NJ).
- [34] Villadsen, J., Nielsen, J., Liden, G. (2011). Bioreaction Engineering Principles. Springer, New York.
- [35] Hidayatullah, I.M., Setiadi, T., Kresnowati, M.T.A.P., Boopathy, R. (2020). Xylanase inhibition by the derivatives of lignocellulosic material. *Bioresour. Technol.*, 300, 122740. DOI: 10.1016/j.biortech.2020.122740
- [36] Duarte, L.C., Carvalheiro, F., Neves, I., Girio, F.M. (2005). Effects of Aliphatic Acids, Furfural, and Phenolic Compounds on Debaryomyces hansenii CCMI 941. Appl. Biochem. Biotechnol., 121–124, 413–425. DOI: 10.1007/978-1-59259-991-2_36
- [37] Sampaio, F.C., Torre, P., Passos, F.M.L., De Moraes, C.A., Perego, P., Converti, A. (2007). Influence of inhibitory compounds and minor sugars on xylitol production by Debaryomyces hansenii. *Appl. Biochem. Biotechnol.*, 136, 165–181. DOI: 10.1007/BF02686021
- [38] Wannawilai, S., Chisti, Y., Sirisansaneeyakul, S. (2017). A model of furfural-inhibited growth and xylitol production by Candida magnoliae TISTR 5663. Food Bioprod. Process., 105, 129–140. DOI: 10.1016/j.fbp.2017.07.002
- [39] Pappu, J.S.M., Gummadi, S.N. (2016). Modeling and simulation of xylitol production in bioreactor by Debaryomyces nepalensis NCYC 3413 using unstructured and artificial neural network models. *Bioresour. Technol.*, 2 2 0 , 4 9 0 - 4 9 9 . D O I : 10.1016/j.biortech.2016.08.097
- [40] Dominguez, J.M., Cheng, S.G., Tsao, G.T. (1997). Production of xylitol from D-xylose by debaryomyces hansenii. Appl. Biochem. Biotechnol., 63–65, 117–127. DOI: 10.1007/BF02920418
- [41] Breuer, U., Harms, H. (2006). Debaryomyces hansenii An extremophilic yeast with biotechnological potential. *Yeast*, 23, 415–437. DOI: 10.1002/yea.1374

- [42] Converti, A., Perego, P., Domínguez, J.M., Silva, S.S. (2001). Effect of temperature on the microaerophilic metabolism of Pachysolen tannophilus. *Enzyme Microb. Technol.*, 28, 339–345. DOI: 10.1016/S0141-0229(00)00330-6
- [43] Bajpai, P. (2014). Chapter 2 Xylan: Occurrence and Structure. In: Bajpai, P. (ed.) Xylanolytic Enzymes. pp. 9–18. Academic Press, Amsterdam.
- [44] Mardawati, E., Maharani, N., Wira, D.W., Harahap, B.M., Yuliana, T., Sukarminah, E. (2020). Xylitol Production from Oil Palm Empty Fruit Bunches (OPEFB) Via Simultaneous Enzymatic Hydrolysis and Fermentation Process. J. Ind. Inf. Technol. A g r i c . , 2 , 2 9 3 6 . D O I : 10.24198/jiita.v2i1.25064
- [45] Öhgren, K., Bura, R., Lesnicki, G., Saddler, J., Zacchi, G. (2007). A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover. *Process Biochem.*, 42, 834–839. DOI: 10.1016/j.procbio.2007.02.003

- [46] Mardawati, E., Trirakhmadi, A., Kresnowati, M., Setiadi, T. (2017). Kinetic study on Fermentation of xylose for The Xylitol Production. J. Ind. Inf. Technol. Agric., 1, 1– 6. DOI: 10.24198/jiita.v1i1.12214
- [47] Josefa K.M., Alejandro, B., Juan P.A., Isabel, O., Andres, V., Mario, A. (2018). Biotechnological Production of Xylitol from Oil Palm Empty Fruit Bunches Hydrolysate. Adv. J. Food Sci. Technol., 16, 134–137. DOI: 10.19026/ajfst.16.5945
- [48] Zhou, J., Liu, Y., Shen, J., Zhang, R., Tang, X., Li, J., Wang, Y., Huang, Z. (2015). Kinetic and thermodynamic characterization of a novel low-temperature-active xylanase from Arthrobacter sp. GN16 isolated from the feces of Grus nigricollis. *Bioengineered.*, 6, 111–114. DOI: 10.1080/21655979.2014.1004021